Predicting the Sensitivity of Oviparous Vertebrates to Dioxin-Like Compounds Based on In Vitro Activation of the AHR
• Diversity in sensitivity to contaminants presents a major challenge to Ecological Risk Assessment

• Fishes are the largest and most diverse group of vertebrates (>30,000)

• Around 1,000 species known in Canada and more known in the USA
• Include Dioxins, Furans, and PCBs
• Found in complex mixtures in the environment
• Different DLCs have vastly different potencies
• Fish being among most sensitive organisms
Biological Effects

- Craniofacial
- Cardiovascular
- Skeletal
- Edema
- Mortality

- Hepatotoxicity
- Fin necrosis
- Wasting syndrome
- Immune suppression
- Reproductive failure
Great differences in sensitivity both among species and taxa

> 1,000-fold

~50-fold

???

~200-fold
Great differences in species sensitivity

Most Sensitive
- Lake Trout
- Brook Trout
- Crucian Carp
- Fundulus
- Red Seabream
- Rainbow Trout
- Fathead Minnow
- Lake Sturgeon
- Channel Catfish
- Lake Herring
- Japanese Medaka
- White Sucker
- Northern Pike
- Zebrafish

Least Sensitive
- Pallid Sturgeon
- Shovelnose Sturgeon

~200-fold difference in embryo-lethality

LD50 (pg TCDD/g-egg)
Biological Effects

• All effects of dioxin-like compounds believed to be mediated through activation of the **Aryl Hydrocarbon Receptor (AhR)** = Molecular Initiation Event (MIE)

• Fish express two AhRs (AhR1 and AhR2)

[Diagram of AhR activation and trans-activation]
Sensitivity of birds to dioxins, furans, and PCBs is predicted by AHR1

The molecular basis for differential dioxin sensitivity in birds: Role of the aryl hydrocarbon receptor

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and related halogenated aromatic hydrocarbons (HAHs) are highly toxic to most organisms. Some of the most dramatic differences in sensitivity to HAHs occur in birds. The domestic chicken (Gallus gallus), an important agricultural species, is highly sensitive to TCDD, whereas the tern (Sterna hirundo) and black-footed albatross (Phoebastria nigripes) are highly resistant. These differences in sensitivity are due to different affinities for the aryl hydrocarbon receptor (AhR) binding sites: high affinity for the chicken and conformational defects in the tern and black-footed albatross.

Sequence and In Vitro Function of Chicken, Ring-Necked Pheasant, and Japanese Quail AHR1 Predict In Vivo Sensitivity to Dioxins

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Objectives

Investigate differences in the activation of the AHR (AHR1 and AHR2) by DLCs among fishes:

> Sensitivity
> Affinity of AHR
Objectives

Develop an *in vitro* tool to allow predicting the relative sensitivity of *endangered species* that cannot easily be studied *in vivo* due to practical, ethical, and ecological reasons.
Most Sensitive

- Lake Trout
- Brook Trout
- Crucian Carp
- Fundulus
- Red Seabream
- Rainbow Trout
- Fathead Minnow
- Lake Sturgeon
- Channel Catfish
- Lake Herring
- Japanese Medaka

Least Sensitive

- White Sucker
- Northern Pike
- Zebrafish
- Pallid Sturgeon
- Shovelnose Sturgeon

- Total of 8 species spanning ~40-fold difference in LD50 of embryos
- Acquire AHR1 and AHR2 information
Luciferase Reporter Gene Assay (LRG)
AhR1 Activation

Most Sensitive
- Lake Trout
- Brook Trout
- Crucian Carp
- Fundulus
- Red Seabream
- Rainbow Trout
- Fathead Minnow
- Lake Sturgeon
- Channel Catfish
- Lake Herring
- Japanese Medaka

Least Sensitive
- White Sucker
- Northern Pike
- Zebrafish
- Pallid Sturgeon
- Shovelnose Sturgeon

![Graphs showing the relative sensitivity of different fish species to AhR1 activation, with LD50 values for Fathead Minnow, Japanese Medaka, Northern Pike AHR1a, and Northern Pike AHR1b.]
No linear relationship between LD50 of embryos and EC50 of AHR1 among fishes.
AhR2 Activation

**Most Sensitive**
- Lake Trout
- Brook Trout
- Crucian Carp
- Mummichog
- Red Seabream
- Rainbow Trout
- Fathead Minnow
- Channel Catfish
- Lake Herring
- Japanese Medaka Fish
- White Sucker
- Northern Pike
- Zebrafish

**Least Sensitive**
- Pallid Sturgeon
- Shovelnose Sturgeon

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**Graphs:**
- Lake Trout AHR2a: EC50 = 0.29 nM, LD50 = 72 pg/g egg
- Lake Trout AHR2b: EC50 = 0.066 nM, LD50 = 72 pg/g egg
- Brook Trout AHR2a: EC50 = 3.7 nM, LD50 = 200 pg/g egg
- Fathead Minnow AHR2: EC50 = 0.65 nM, LD50 = 539 pg/g egg
- Japanese Medaka AHR2: EC50 = 4.6 nM, LD50 = 1110 pg/g egg
- White Sucker AHR2: EC50 = 5.7 nM, LD50 = 1890 pg/g egg
- Northern Pike AHR2a: EC50 = 0.30 nM, LD50 = 2460 pg/g egg
- Northern Pike AHR2b: EC50 = 11 nM, LD50 = 2460 pg/g egg

**LD50 (pg/g egg):**
0 2,000 4,000 6,000 8,000 10,000 12,000 14,000
Highly significant, linear relationship between LD50 of embryos and EC50 of AHR2 among fishes

$R^2 = 0.97$

$p = < 0.0001$
Relative Sensitivity?

Most Sensitive

- Lake Trout
- Brook Trout
- Crucian Carp
- Mummichog
- Red Seabream
- Rainbow Trout
- Fathead Minnow
- Channel Catfish
- Lake Herring
- Japanese Medakafish
- White Sucker
- Northern Pike
- Zebradish

Least Sensitive

- Pallid Sturgeon
- Shovelnose Sturgeon

Where do endangered white sturgeon fit?
<table>
<thead>
<tr>
<th>Dioxin-like Compound</th>
<th>AHR2 EC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
<th>Predicted LD&lt;sub&gt;50&lt;/sub&gt; (pg/g-egg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCDD</td>
<td>0.070</td>
<td>82</td>
</tr>
<tr>
<td>PeCDF</td>
<td>0.034</td>
<td>51</td>
</tr>
<tr>
<td>TCDF</td>
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<td>84</td>
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<tr>
<td>PCB 126</td>
<td>1.8</td>
<td>842</td>
</tr>
<tr>
<td>PCB 77</td>
<td>38</td>
<td>6,743</td>
</tr>
</tbody>
</table>

**White Sturgeon (Acipenser transmontanus)**
<table>
<thead>
<tr>
<th>Dioxin-like Compound</th>
<th>AHR2 EC$_{50}$ (nM)</th>
<th>Predicted LD$_{50}$ (pg/g-egg)</th>
<th>Measured LD$_{50}$ (pg/g-egg nominal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCDD</td>
<td>0.070</td>
<td>82</td>
<td>105</td>
</tr>
<tr>
<td>PeCDF</td>
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<td>&lt; 4,000</td>
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<tr>
<td>PCB 77</td>
<td>38</td>
<td>6,743</td>
<td>238,400</td>
</tr>
</tbody>
</table>
Can we predict sensitivity to other vertebrates?

Fish AHR2
Slope = 0.67
y-intercept = 0.22

Bird AHR1
Slope = 0.84
y-intercept = 0.38
Relationship Between AhR Activation and Embryo Toxicity

logLD50 (pmol/g-egg) vs logEC50 (nM)

R² = 0.88
p = < 0.0001
Activation of the AhR leading to embryo toxicity in oviparous vertebrates

MIE = Activation of the AhR

AO = Teratogenicity
Incorporating a greater diversity of fishes for furans and PCBs

- Japanese Medaka
- Zebrafish
- White Sturgeon
- Lake Sturgeon
- Eel
• Incorporating a greater diversity of fishes for furans and PCBs
• Investigating whether AHR1 of amphibians follows the same predictive relationship
• Incorporating a greater diversity of fishes for furans and PCBs
• Investigating whether AHR1 of amphibians follows the same predictive relationship
• Investigate whether sensitivity to other agonists of the AHR, such as polycyclic aromatic hydrocarbons (PAHs), can be predicted from AHR binding

Benzo[a]pyrene
Ongoing Work

- Incorporating a greater diversity of fishes for furans and PCBs
- Investigating whether AHR1 of amphibians follows the same predictive relationship
- Investigate whether sensitivity to other agonists of the AHR, such as polycyclic aromatic hydrocarbons (PAHs), can be predicted from AHR binding
- Identify structural elements of the AHR that drive differences in binding and therefore determine sensitivity \textit{in vivo}
Acknowledgements

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Questions ??
Highly significant, linear relationship between LD50 of embryos and EC50 of AHR1 or AHR2 among birds and fishes across dioxin-like compounds.

$R^2 = 0.87$
$p = < 0.0001$
Fish vs Birds

Not significantly different
Slope \( p = 0.11 \)  \( y \)-intercept \( p = 0.052 \)